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REVIEW ARTICLE

ALK in Lung Cancer: Past, Present, and Future

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ABSTRACT

In 2007, scientists discovered that anaplastic lymphoma kinase (*ALK*) gene rearrangements are present in a small subset of non–small-cell lung cancers. *ALK*-positive cancers are highly sensitive to small-molecule ALK kinase inhibitors, such as crizotinib. Phase I and II studies of crizotinib in *ALK*-positive lung cancer demonstrated impressive activity and clinical benefit, leading to rapid US Food and Drug Administration approval in 2011. Although crizotinib induces remissions and extends the lives of patients, cures are not achieved as resistance to therapy develops. In this review, we will discuss the history of this field, current diagnostic and treatment practices, and future challenges and opportunities to advance outcomes for patients with *ALK*-positive lung cancers.

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INTRODUCTION

The last decade has witnessed a major paradigm shift in the treatment of advanced non–small-cell lung cancer (NSCLC). Although histologic subtype is clearly an important factor in selecting among standard cytotoxic chemotherapies, we now recognize that the presence of key oncogenic alterations, such as activating mutations and chromosomal rearrangements, predicts responsiveness to selective targeted therapies. In lung cancer, this paradigm was first established with the discovery that epidermal growth factor receptor (*EGFR*) mutations are associated with sensitivity to the EGFR tyrosine kinase inhibitor (TKI) gefitinib. More recently, NSCLCs harboring translocations in anaplastic lymphoma kinase (*ALK*) have demonstrated remarkable sensitivity to the ALK kinase inhibitor crizotinib.

ALK gene rearrangements were only first reported in NSCLC in 2007,[1,2] yet significant advances have rapidly culminated in the recent accelerated approval of the ALK inhibitor crizotinib by the US Food and Drug Administration (FDA). Herein, we review the remarkably short but substantial history of ALK in lung cancer. This review starts with a brief examination of the discovery and biology of *ALK* rearrangements and then summarizes the results of key clinical trials of crizotinib, which have validated ALK as a therapeutic target and led to the current standard of care for patients with advanced, *ALK*-positive NSCLC. In the final section, we examine ongoing research into mechanisms of crizotinib resistance. Acquired resistance has emerged as the major hurdle in the treatment of *ALK*-positive NSCLC, because patients typically relapse within 1 to 2 years of starting therapy. Deciphering the complexity of resistance mechanisms will be critical to effectively guiding the development of future therapeutic strategies aimed at overcoming resistance in the clinic.

THE PAST: DISCOVERY OF *ALK* REARRANGEMENTS AS ONCOGENIC DRIVERS IN HUMAN CANCERS

ALK was first discovered more than 17 years ago as a fusion oncogene with nucleophosmin (*NPM*) in a subset of anaplastic large-cell lymphomas (ALCLs).[3] *NPM-ALK* arises from a translocation involving chromosome 2p, which harbors *ALK*, and chromosome 5q, which harbors *NPM*. Other ALK fusion proteins have also been identified in ALCL, including TPM3-ALK and TFG-ALK.[4,5] *ALK* translocations were next discovered approximately 11 years ago in a subset of inflammatory myofibroblastic tumors (IMTs).[4] However, it was not until 4.5 years ago that interest in ALK surged after a pivotal publication by a team led by Hiroyuki Mano, MD, PhD, describing the discovery of a novel ALK fusion—echinoderm microtubule-associated protein-like 4 (*EML4*)–*ALK*—as a somatic gene rearrangement found in a small percentage of Japanese lung cancers.[1] *EML4-ALK* fusions result from small inversions within chromosome 2p that fuse differing portions of the *EML4* gene with a portion of the *ALK* gene. EML4-ALK is the predominant ALK fusion in lung cancer, although several other ALK fusions have now been reported, including KIF5B-ALK, TFG-ALK, and KLC1-ALK (Fig 1).[2,6,7] In almost all

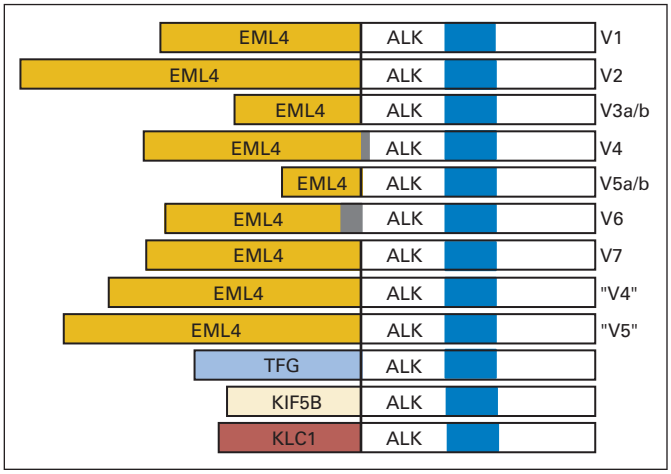

Fig 1. Schematic diagram depicting some of the anaplastic lymphoma kinase (ALK) fusion proteins identified in non–small-cell lung cancer (NSCLC). Echinoderm microtubule-associate protein-like 4 (EML4) –ALK variants are the predominant ALK fusions in NSCLC. More than 20 EML4-ALK variants have been identified, nine of which are shown here. Three other partner proteins have been identified in NSCLC: TFG, KIF5B, and KLC1. Three different KIB5B-ALK variants have been identified (not shown). The blue rectangles within each fusion protein symbolize the ALK tyrosine kinase domain. Adapted.[7a]

of the known *ALK* rearrangements, including *EML4-ALK*, the genomic breakpoint within the *ALK* gene is conserved.

The wild-type (or nonrearranged) *ALK* gene encodes an orphan receptor tyrosine kinase (RTK) that is believed to play a role in the development of the nervous system.[8] In the adult, expression of *ALK* is largely restricted to certain neuronal cells. At the cellular level, ALK regulates canonical signaling pathways that are shared with other RTKs, including RAS–mitogen-activated protein kinase (MAPK), phosphoinositide 3-kinase (PI3K) –AKT, and JAK-STAT pathways. In the case of *ALK* rearrangements, 5′ end partners like *NPM* and *EML4* are fused to the intracellular tyrosine kinase domain of *ALK*, leading to aberrant expression of the ALK fusions in the cytoplasm. In addition, the domains in the partner proteins promote dimerization and oligomerization of the fusion proteins, leading to constitutive activation of ALK kinase and its downstream signaling pathways. This results in uncontrolled cellular proliferation and survival.

Numerous studies have demonstrated that ALK fusion proteins are oncogenic and drive transformation both in vitro and in vivo. In the original report describing *ALK* rearrangements in NSCLC, mouse 3T3 cells transfected with a plasmid encoding *EML4-ALK* formed foci in soft agar and large subcutaneous tumors in nude mice. In contrast, a kinase-dead version of EML4-ALK—K589M—failed to induce foci or tumors, suggesting that the kinase activity of EML4-ALK is critical for its oncogenic potential.[1] Similarly, in follow-up reports, investigators generated transgenic mice expressing *EML4-ALK* under the control of a lung-specific promoter. As shown by serial computed tomography scans and confirmed histologically, all transgenic animals developed numerous lung adenocarcinomas expressing the ALK fusion protein.[9,10] Thus, EML4-ALK is sufficient to induce lung tumorigenesis in vivo.

Preclinical studies have demonstrated that cancers with *ALK* translocations are dependent on continued ALK signaling for growth and survival.[11] This dependency is commonly referred to as oncogene addiction, and in the settings of addiction to RTKs, this occurs when RAS-MAPK and PI3K-AKT signaling are controlled solely by an RTK like ALK or EGFR. Inhibition of the RTK leads to suppression of these signaling pathways, resulting in cell growth arrest and apoptosis. As an example, the transgenic mice harboring EML4-ALK–expressing lung adenocarcinomas were treated with either with vehicle or with a small-molecule ALK inhibitor. Control animals showed enlarging lung tumors over approximately 3.5 weeks. In contrast, those animals treated with the ALK inhibitor showed marked tumor regression over the same time interval.[9] These results suggest that ALK-driven lung cancers are addicted to ALK and highly sensitive to ALK inhibition.

THE PRESENT: EFFICACY OF CRIZOTINIB IN TREATING PATIENTS WITH ADVANCED, *ALK*-POSITIVE NSCLC

In the clinic, early-phase studies of crizotinib validated ALK as a therapeutic target. Most of our clinical experience with crizotinib is derived from patients with advanced, *ALK*-positive lung cancer. These patients comprise approximately 4% of all patients with NSCLC. By comparison, *KRAS* mutations comprise almost one quarter of NSCLCs, and *EGFR* mutations, which can be effectively targeted by EGFR inhibitors such as gefitinib and erlotinib, are found in 10% to 15% of NSCLCs. Two other targets of crizotinib—ROS1 and c-MET—are even less common than ALK; each present in 1% to 2% of NSCLCs.[12] Nevertheless, because lung cancer is such a prevalent malignancy, the 4% of patients with ALK lung cancer represent potentially 40,000 new cases worldwide each year.

ALK rearrangement has been associated with several distinctive clinicopathologic features.[13,14] One of the most important is absence of smoking history. At Massachusetts General Hospital, close to 15% of lung cancers from patients with a never-smoking history are ALK positive. Among patients with any smoking history, only 2% are ALK positive. Conversely, among patients with *ALK*-positive lung cancer, more than 90% are never- or light smokers (light smoking is defined as $\leq$ 10 pack-years). Other important features associated with *ALK*-positive lung cancers include younger age at diagnosis, adenocarcinoma histology, and absence of other oncogenic drivers.

The role of crizotinib in *ALK*-positive lung cancer was first evaluated in an international, multicenter phase I study.[15] Crizotinib was originally designed and synthesized at Pfizer as a small-molecule TKI targeting c-MET.[16] Subsequently, the selectivity of crizotinib was evaluated using an Upstate kinase selectivity screen as well as cell-based enzymatic assays. Crizotinib was found to be most potent against c-MET but also active against a handful of other RTKs including ALK and ROS1. Fortuitously, the dose-escalation portion of the phase I study of crizotinib was already in progress when EML4-ALK was first reported in lung cancer. Within a few months, a diagnostic fluorescence in situ hybridization (FISH) assay was developed (Fig 2), and soon thereafter, the first two patients with *ALK*-positive lung cancer were enrolled in dose escalation. Both patients experienced a significant improvement in disease-related symptoms. Additional patients with *ALK*-positive lung cancers were then enrolled in a dose-expansion cohort at the maximum-tolerated dose.[15]

Preliminary efficacy and safety data on the first 82 *ALK*-positive patients enrolled onto the phase I study were published in October 2010, and the data were recently updated. All patients had advanced, *ALK*-positive NSCLC, and most patients had received at least one prior line of chemotherapy. In general, most of these patients responded to crizotinib, some with remarkable responses (Fig 3).

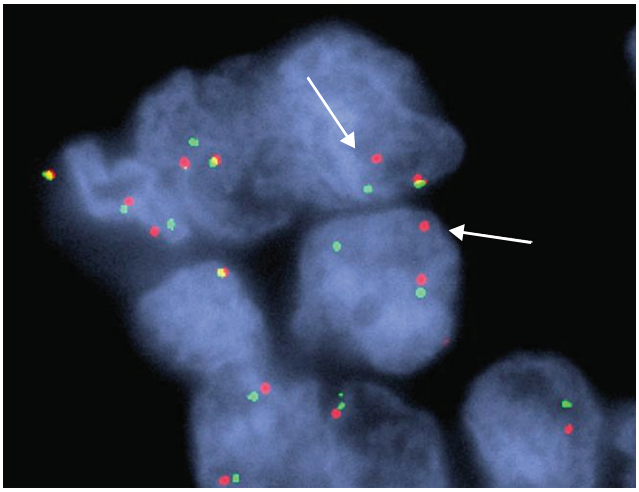

Fig 2. Fluorescence in situ hybridization assay for diagnosing anaplastic lymphoma kinase (*ALK*) rearrangement. In this assay, DNA probes with attached fluorescent dyes (red and green) flank the highly conserved breakpoint within the *ALK* gene. Separation of the probes because of *ALK* gene rearrangement results in splitting of the red and green signals (arrows). Reprinted with permission.[13]

Among 143 evaluable patients, the objective response rate (ORR) was 60.8%. The median duration of response was 49.1 weeks, and the median progression-free survival (PFS) was 9.7 months.[17] By comparison, standard single-agent chemotherapies in previously treated patients with advanced unselected NSCLC had traditionally led to response rates of 10% or less and a median PFS of 2 to 3 months.[18,19]

Similar impressive clinical activity has been observed in an ongoing global phase II study of crizotinib in advanced, *ALK*-positive NSCLC. At the time of FDA submission, the ORR among 133 evaluable patients was 51.1%. Updated efficacy data from the phase II study were presented at the 48th Annual Meeting of the American Society of Clinical Oncology (ASCO) this year. As of January 2012, 901 patients had been dosed on this study. In the mature study population of 259 patients, the ORR was 59.8%, and the median PFS was 8.1 months.[20] In both studies, crizotinib was well tolerated, with mild, primarily grade 1 adverse events, including visual disturbance, nausea/vomiting, diarrhea, constipation, fatigue, and peripheral edema.

On the basis of the response rates demonstrated in the phase I and II studies, along with its safety profile, crizotinib was granted accelerated approval by the FDA last August, 4 years after the first report of *ALK* rearrangement in lung cancer. Crizotinib was approved in conjunction with an FDA-approved diagnostic assay, currently the Vysis ALK Break-Apart FISH Probe Kit (Abbott Molecular, Des Plaines, IL). Of note, ALK immunohistochemistry (IHC) may also prove effective in the future, because ALK expression is restricted to those lung cancers with *ALK* gene rearrangement, but the use of IHC to identify patients for treatment with crizotinib has not yet been validated.[21] Current guidelines from the National Comprehensive Cancer Network (NCCN) recommend testing all patients with advanced, nonsquamous NSCLC for both *EGFR* mutation and *ALK* rearrangement. Because the FDA label does not specify a requirement for previous treatment, newly diagnosed, *ALK*-positive patients can be prescribed crizotinib in the first-line setting. In fact, despite the absence of randomized data, the NCCN does recommend crizotinib as first-line therapy in advanced, *ALK*-positive NSCLC. This recommendation to use crizotinib as first-line therapy is largely based on our experience with *EGFR*-mutant NSCLC, where the results of five randomized studies have now proven the superiority of EGFR inhibitors versus platinum doublets in prolonging PFS in the first-line setting.[22-26]

At present, the results of two international, randomized phase III studies of crizotinib are eagerly awaited. The second-line trial (PROFILE 1007) is comparing crizotinib with single-agent pemetrexed or docetaxel in patients with advanced, *ALK*-positive NSCLC who have received one prior platinum-based chemotherapy regimen. This trial enrolled its first patient in early 2010 and surpassed its target enrollment of 318 earlier this year. Results will be available before the end of 2012. The first-line trial (PROFILE 1014) is comparing crizotinib with a platinum/pemetrexed combination in newly diagnosed patients with advanced, *ALK*-positive NSCLC. The primary end point of both studies is PFS, with overall survival (OS) as a secondary end point. The OS benefit of crizotinib will likely be confounded in both

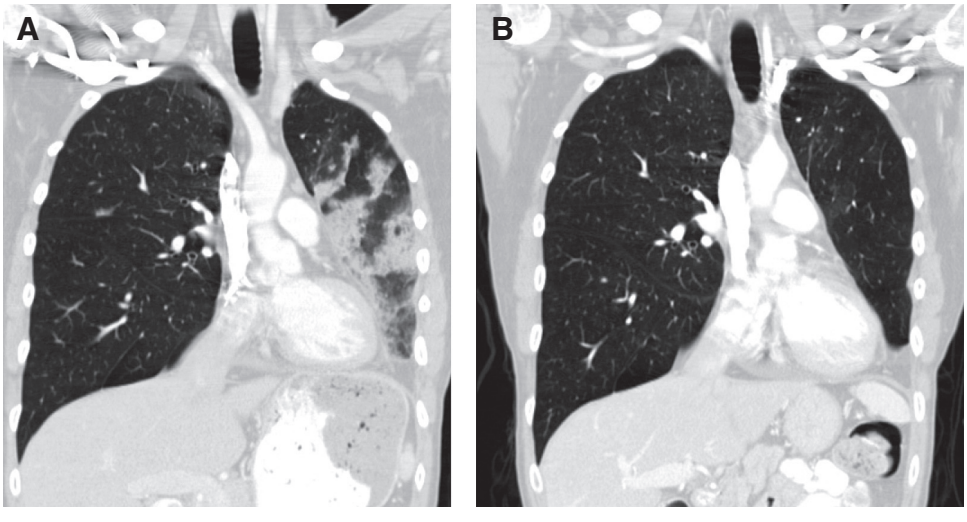

Fig 3. Typical clinical response of an anaplastic lymphoma kinase (*ALK*) –positive patient to crizotinib. (A) Before crizotinib; (B) after 7 weeks of crizotinib. Reprinted with permission.[15]

trials because of crossover; crossover is required as it would be unethical to have a randomized trial that deprived an *ALK*-positive patient of crizotinib. However, in a retrospective OS analysis of patients with *ALK*-positive NSCLC comparing those who received crizotinib in the phase I trial with those who never received crizotinib, treatment with crizotinib was associated with a substantial prolongation in OS.[27]

In both randomized phase III studies of crizotinib, pemetrexed is included in the standard chemotherapy arm. Two small retrospective studies have suggested that pemetrexed-based chemotherapy may be particularly active in *ALK*-positive NSCLC.[28,29] Both studies reported a median PFS or median time to progression of approximately 9 months. However, these studies were small, single-institution series with only 15 patients in one study and 19 patients in the other. In a large multicenter retrospective analysis (N = 121), we recently found that *ALK*-positive patients did not have a longer PFS with pemetrexed-based chemotherapy compared with ALK-negative controls, except in the setting of first-line platinum/pemetrexed combinations.[30] As an example, the median PFS with single-agent pemetrexed administered as second- or third-line therapy was only 4.4 months, significantly shorter than those in previous reports. Furthermore, within the subset of patients with a never- or light smoking history, PFS with all pemetrexed regimens, including first-line platinum/pemetrexed, was remarkably similar between *ALK*-positive and *ALK*-negative patients. Thus, never- or light smoking status, rather than *ALK* rearrangement, may be a predictor of pemetrexed response.[30]

THE FUTURE: OVERCOMING CRIZOTINIB RESISTANCE

Despite the marked antitumor activity of crizotinib, ALK-driven cancers invariably become resistant to crizotinib. In the case of *ALK*-positive lung cancer, as well as *EGFR* mutant lung cancer, resistance develops on average within the first year or two of TKI therapy. However, there is marked heterogeneity in the duration of benefit, ranging from a few months to several years. Acquired resistance ultimately limits the clinical benefit of TKIs, and it clearly represents the most pressing challenge in the field of targeted therapies. Our discussion will focus only on acquired, as opposed to intrinsic, resistance, because the latter is much less common and still poorly understood.

Mechanisms

The phenomenon of acquired resistance has been extensively studied in other oncogene-addicted cancers, such as *EGFR*-mutant lung cancer treated with EGFR TKIs and *BRAF*-mutant melanoma treated with specific BRAF inhibitors. In general, mechanisms of acquired drug resistance reside in one of two classes. First, the target gene itself can be altered either by mutation or by amplification, limiting the ability of the drug to inhibit the kinase. In the presence of the drug, the kinase remains active and drives aberrant signaling. Examples of this class of resistance include the known gatekeeper mutations T790M in *EGFR* and T315I in *BCR-ABL*.[31-33] Second, alternative signaling pathways or so-called bypass tracks can be activated in resistant cells, bypassing the need for signaling from the target. As an example, in 5% to 10% of EGFR TKI–resistant cases, resistance is mediated by focal amplification of *c-MET*.[34] c-MET activates downstream signaling independently of EGFR, allowing resistant cells to grow despite EGFR inhibition.

Several studies have now been published on crizotinib resistance in *ALK*-positive lung cancer, including one series from Massachusetts General Hospital (N = 18) and one series from University of Colorado (N = 11).[35,36] In up to one third of relapsing patients, crizotinib resistance is mediated by secondary resistance mutations located in the *ALK* TK domain. Across all studies to date, the most commonly identified resistance mutation is the gatekeeper mutation L1196M,[37-39] analogous to *EGFR* T790M and *BCR-ABL* T315I. Reported in a total of four patients with acquired resistance to crizotinib, the L1196M amino acid substitution is believed to hinder TKI binding through steric hindrance. However, in contrast to EGFR TKI–resistant lung cancer, in which T790M is essentially the sole resistance mutation observed in the clinic,[40] there are many different resistance mutations distributed throughout the *ALK* TK domain. For example, the G1269A substitution, identified in two patients in the Colorado series,[36] lies directly in the ATP-binding pocket. Two mutations, G1202R and S1206Y, are located in the solvent-exposed region of the kinase domain and may decrease the binding affinity of crizotinib.[35] In contrast, several resistance mutations, like the 1151 threonine insertion, are predicted to lie farther away from the ATP-binding site, but through conformational changes they may also compromise crizotinib binding.[35]

In addition to *ALK* resistance mutations, amplification of the *ALK* fusion gene has also been reported in a small number of crizotinib-resistant tumors, with and without concurrent *ALK* mutation.[35,36] Amplification has been established using the standard breakapart FISH assay for *ALK* rearrangement.[13] Interestingly, in cell-line models of acquired resistance to crizotiinib, amplification of *EML4-ALK* has been shown to mediate crizotinib resistance.[38] These cell-line models were generated by culturing a sensitive cell line harboring EML4-ALK (H3122) in increasing concentrations of crizotinib until resistant clones emerged. The fully resistant cells (resistant to 1 μmol/L of crizotinib) were found to harbor both the gatekeeper L1196M mutation as well as *EML4-ALK* amplification. However, the partially resistant predecessors of the fully resistant clones (resistant to 300 nmol/L of crizotinib) had amplification without mutation. This observation suggests that amplification alone is sufficient to mediate resistance to intermediate concentrations of crizotinib.[38]

At our institution, close to one third of resistant specimens have been found to harbor *ALK* resistance mutations or fusion gene amplification. This suggests that among the remaining two thirds of patients, there are likely other distinct mechanisms of resistance, such as activation of bypass tracks. In crizotinib-resistant tumors, several distinct bypass tracks mediating resistance have been reported. The first is EGFR, which has been reported in several independent studies.[35,41] In the Massachusetts General Hospital series, 17 of 18 resistant specimens demonstrated some degree of EGFR activation based on IHC staining for phosphorylated EGFR. In approximately one half of evaluable cases, there was an increase in EGFR activation in the resistant cancer compared with the corresponding sensitive sample. The precise mechanism by which EGFR is activated is unknown, although in vitro studies suggest that EGFR and some of its ligands may be upregulated.[35] Importantly, inhibition of EGFR resensitized these resistant cell lines to crizotinib. Of note, *EGFR* mutations have not been identified in any resistant, *ALK*-positive tumor specimens,[35,36] so mutational activation is unlikely to account for the increase in phospho-EGFR.

The second bypass track activated in crizotinib-resistant disease is c-KIT. In two of the 18 resistant specimens in the Massachusetts General Hospital series, we discovered high-level *c-KIT* gene amplification by FISH.[35] Overexpression of c-KIT was confirmed by IHC. In addition, by IHC, there was also increased expression of the c-KIT ligand stem-cell factor (SCF) in the stromal cells of the solid component of the resistant specimens. Laboratory experiments confirmed that c-KIT overexpression required SCF to promote resistance, and resistance could be overcome by combined imatinib and crizotinib treatment. Interestingly, in one of the resistant cases with *c-KIT* amplification, an adjacent histologically distinct bronchioloalveolar component of the resistant specimen showed no evidence of c-KIT or SCF overexpression, but it did show increased phospho-EGFR staining compared with the precrizotinib sample, raising the possibility of multiple bypass tracks activated in an individual patient.[35]

Finally, in the Colorado series of 11 crizotinib-resistant tumors, two were found to harbor a *KRAS* mutation, suggesting that KRAS activation could represent a potential resistance mechanism.[36] The significance of this finding is uncertain, because expression of oncogenic *KRAS* in sensitive H3122 cells did not induce resistance to crizotinib. In addition, we have recently examined 22 crizotinib-resistant tumors using the SNaPshot genotyping platform (Applied Biosystems, Foster City, CA)[42] and have identified no cases with *KRAS* mutation.

New Treatment Strategies

Understanding the mechanistic bases for acquired resistance is essential to developing strategies to overcome (or delay) resistance in the clinic. Figure 4 depicts a summary of the known resistance mechanisms in *ALK*-positive NSCLC. For the one third of patients in whom crizotinib resistance is mediated by *ALK* mutation or amplification, cancers are still addicted to ALK, and therefore, next-generation ALK inhibitors may be effective in reinducing remissions. Hsp90 inhibitors may also be active in this setting, because ALK fusion proteins, including those with resistance mutations, are known Hsp90 clients.[38,43]

Next-generation ALK inhibitors are structurally distinct from crizotinib, generally more potent than crizotinib, and are currently being developed in the clinic to overcome crizotinib resistance. To date, at least four new ALK inhibitors are currently in early-phase studies, including LDK378 (Novartis, Basel, Switzerland), AP26113 (ARIAD Pharmaceuticals, Cambridge, MA), AF802 (Chugai Pharmaceutical, Tokyo, Japan), and ASP3026 (Astellas Pharma, Tokyo, Japan); many others are also in development. Preliminary safety and efficacy results for one of these—LDK378—were presented at the 48th ASCO Annual Meeting.[44] To date, among 26 *ALK*-positive patients who had previously relapsed with crizotinib and who received LDK378 $\geq$ 400 mg, the ORR was 81%. The median duration of response is not yet known. In addition, the time interval between crizotinib and LDK378 was not reported. Nevertheless, the marked activity observed with LDK378 in *ALK*-positive NSCLC suggests that there may be a role for more potent ALK inhibition in treating crizotinib-resistant disease.

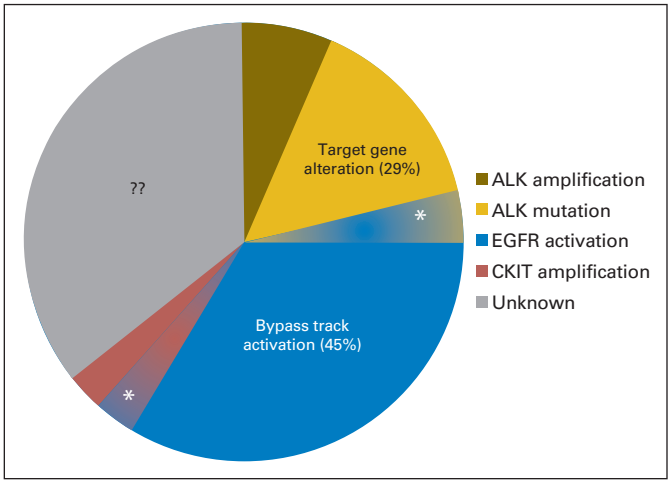

Fig 4. Summary of crizotinib resistance mechanisms in anaplastic lymphoma kinase (*ALK*) –positive non–small-cell lung cancer. Question marks indicate patients in whom the mechanism of resistance is unknown. EGFR, epidermal growth factor receptor. (*) Patients with more than one resistance mechanism.

The discovery of multiple different *ALK* resistance mutations may have important clinical implications if they are associated with differential sensitivity to different ALK inhibitors. This possibility has been studied in Ba/F3 cells engineered to express either wild-type *EML4-ALK* or *EML4-ALK* harboring one of five different resistance mutations.[35] Ba/F3 cells expressing wild-type or mutated forms of *EML4-ALK* were treated with a panel of different ALK inhibitors, including many of the clinically available ALK inhibitors. Relative to wild-type *EML4-ALK*, mutant forms of *EML4-ALK* were resistant to crizotinib but showed different degrees of crizotinib resistance depending on the mutation. Similarly, with each of the next-generation ALK inhibitors, potency seems to vary widely depending on the individual resistance mutation. For example, *EML4-ALK* containing the resistance mutation G1269A was highly sensitive to several of the second-generation ALK inhibitors, whereas the solvent front mutation G1202R conferred high-level resistance to almost all of the ALK TKIs tested. Overall, these findings suggest that second-generation ALK inhibitors are not equivalent and have differential potencies against different resistance mutations.[35] In the clinic, this could translate into the need to develop multiple second-generation ALK inhibitors to overcome specific subsets of resistance mutations and induce durable remissions.

Clinical trials of next-generation ALK inhibitors as well as other ALK-targeted therapies are the first step in tackling crizotinib resistance, but single-agent therapy is unlikely to transform the course of disease for the majority of patients who experience relapse with crizotinib. Several studies have already shown that more than one resistance mechanism may be active in an individual patient. For example, in the first case report of crizotinib resistance, the resistant tumor was found to harbor two different resistance mutations, L1196M and C1156Y.[37] As mentioned earlier, a resistant specimen in the Massachusetts General Hospital series showed both c-KIT and EGFR activation, but in two spatially as well as histologically different sections of the specimen.[35] Because we typically biopsy only a single resistant site, we may not even recognize the potential heterogeneity of resistance mechanisms in each patient. Furthermore, the activation of alternative RTKs like EGFR suggests the need for combination strategies like combined ALK and EGFR inhibitors. Indeed, there are currently two ongoing early-phase studies of crizotinib with either erlotinib or dacomitinib (PF-299804). However, even these combination strategies may be too simplistic given the potential multiplicity of bypass tracks beyond EGFR and c-KIT. To substantially advance this field, we may need to develop more sophisticated diagnostic tools to more accurately assess

the full spectrum of resistance mechanisms that exist in an individual patient. In addition, more innovative treatment strategies aimed at preventing the emergence of resistance may be needed. These may include regimens that adopt alternative dosing and scheduling of more complex combinations employed in an alternating or intercalated manner.

DISCUSSION

In conclusion, ALK is now a validated kinase target in lung and other cancers. *ALK*-positive cancers are oncogene addicted and, as a result, highly responsive to crizotinib. However, patients with *ALK*-positive lung cancer invariably relapse with crizotinib as a result of the development of resistance. Approximately one third of resistance may be attributed to alterations in ALK itself, including a diverse array of resistance mutations as well as *ALK* fusion gene amplification. An additional one half of patients may experience activation of bypass tracks such as EGFR or c-KIT. Other bypass tracks as well as other types of resistance mechanisms remain to be discovered and validated. In cell-line studies and in patients, multiple resistance mechanisms can develop simultaneously, highlighting the need for novel combinatorial strategies to overcome crizotinib resistance and further improve the clinical outcome of patients with advanced, *ALK*-positive NSCLC.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
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Final approval of manuscript: All authors

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